Research Article

Photosynthetic response of *Cannabis sativa* L. to variations in photosynthetic photon flux densities, temperature and CO_2 conditions

Suman Chandra[1], Hemant Lata[1], Ikhlas A. Khan[1,2] and Mahmoud A. Elsohly[1,3]

[1]*National Center for Natural Product Research, School of Pharmacy, University of Mississippi, MS-38677, USA.*
[2]*Department of Pharmacognosy, University of Mississippi, MS-38677, USA.*
[3]*Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677, USA.*

ABSTRACT

Effect of different photosynthetic photon flux densities (0, 500, 1000, 1500 and 2000 μmol $m^{-2}s^{-1}$), temperatures (20, 25, 30, 35 and 40 °C) and CO_2 concentrations (250, 350, 450, 550, 650 and 750 μmol mol^{-1}) on gas and water vapour exchange characteristics of *Cannabis sativa* L. were studied to determine the suitable and efficient environmental conditions for its indoor mass cultivation for pharmaceutical uses. The rate of photosynthesis (P_N) and water use efficiency (WUE) of *Cannabis sativa* increased with photosynthetic photon flux densities (PPFD) at the lower temperatures (20-25 °C). At 30 °C, P_N and WUE increased only up to 1500 μmol $m^{-2}s^{-1}$ PPFD and decreased at higher light levels. The maximum rate of photosynthesis ($P_{N\ max}$) was observed at 30 °C and under 1500 μmol $m^{-2}s^{-1}$ PPFD. The rate of transpiration (E) responded positively to increased PPFD and temperature up to the highest levels tested (2000 μmol $m^{-2}s^{-1}$ and 40 ^{0}C). Similar to E, leaf stomatal conductance (g_s) also increased with PPFD irrespective of temperature. However, g_s increased with temperature up to 30 °C only. Temperature above 30 °C had an adverse effect on g_s in this species. Overall, high temperature and high PPFD showed an adverse effect on P_N and WUE. A continuous decrease in intercellular CO_2 concentration (Ci) and therefore, in the ratio of intercellular CO_2 to ambient CO_2 concentration (Ci/Ca) was observed with the increase in temperature and PPFD. However, the decrease was less pronounced at light intensities above 1500 μmol $m^{-2}s^{-1}$. In view of these results, temperature and light optima for photosynthesis was concluded to be at 25-30 °C and ~1500 μmol $m^{-2}s^{-1}$ respectively. Furthermore, plants were also exposed to different concentrations of CO_2 (250, 350, 450, 550, 650 and 750 μmol mol^{-1}) under optimum PPFD and temperature conditions to assess their photosynthetic response. Rate of photosynthesis, WUE and Ci decreased by 50 %, 53 % and 10 % respectively, and Ci/Ca, E and g_s increased by 25 %, 7 % and 3 % respectively when measurements were made at 250 μmol mol^{-1} as compared to ambient CO_2 (350 μmol mol^{-1}) level. Elevated CO_2 concentration (750 μmol mol^{-1}) suppressed E and g_s ~ 29% and 42% respectively, and stimulated P_N, WUE and Ci by 50 %, 111 % and 115 % respectively as compared to ambient CO_2 concentration. The study reveals that this species can be efficiently cultivated in the range of 25 to 30 °C and ~1500 μmol $m^{-2}s^{-1}$ PPFD. Furthermore, higher P_N, WUE and nearly constant Ci/Ca ratio under elevated CO_2 concentrations in *C. sativa*, reflects its potential for better survival, growth and productivity in drier and CO_2 rich environment.
[Physiol. Mol. Biol. Plants 2008; 14(4) : 299-306] *E-mail : suman@olemiss.edu*

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Abbreviations : *PPFD - Photosynthetic photon flux density, P_N - Photosynthesis, Rd – Dark respiration, $P_{N\ max}$ - Maximum rate of photosynthesis, E - Transpiration, g_s - Leaf stomatal conductance, Ci - Leaf internal CO_2 concentration, Ci/Ca - Internal to ambient CO_2 concentration, WUE - Water use efficiency*

The ability of a species to acclimate and adapt to environmental variations is directly/indirectly associated with its ability to modulate photosynthesis and water vapour exchange (Pearcy, 1977; Berry and Downtown, 1982; Stoutjesdijk and Barkman, 1992; Ayuko *et al*., 2008; Dieleman and Meinen, 2008; Kruse *et al*., 2008), which in turn affects biochemical and physiological processes in the leaf and, consequently the physiology and productivity of whole plant. Studies on gas exchange characteristics may provide valuable information on functioning of plants in variable environment. Photosynthesis, being the primary source of carbon and energy, plays a prominent role in the logistics of plant growth. There is a close correlation between

Correspondence and Reprint requests : Suman Chandra

productivity and yield of the plants with their photosynthetic rate, in the given environment, as more than 90% of dry matter of live plants is derived from photosynthetic CO_2 assimilation (Zelitch, 1975). Therefore, photosynthesis is a valuable physiological tool to evaluate the response of plants to environmental stresses and for the rapid selection of plants for a particular environmental condition (Joshi and Palni, 2005; Monclus *et al*., 2006) or selection of suitable environmental conditions for a particular plant species.

Furthermore, elevated CO_2 may increase photosynthetic carbon assimilation and may accelerate plant growth and potentially improve productivity. Indeed, a doubling in CO_2 concentration increases crop yield by 30% or more, in experiments conducted under close environmental conditions such as green houses and growth chambers (Kimball, 1983a, b; 1986; Cure, 1985; Poorter, 1993; Idso and Idso, 1994). Therefore, in the present study, *C. sativa* plants were exposed to a range of CO_2 concentration to understand their response in term of their photosynthetic capacity to the range of elevated CO_2 labels.

Cannabis sativa L. is widely distributed around the world. Originally indigenous to temperate regions of Asia, it now grows in a variety of habitats ranging from sea level in tropical areas to alpine foot hills of Himalayas. *Cannabis* has a long history of the medicinal use in Middle East and Asia, with references as far back as the 6th century B.C. This species was introduced in the Western Europe medicine in the early 19th century A.C. to treat epilepsy, tetanus, rheumatism, migraine, asthma, trigeminal neuralgia, fatigue, and insomnia (Doyle and Spence, 1995; Zuardi, 2006). *C. sativa* contains cannabinoids, a unique class of terpenophenolic compounds, which accumulates mainly in glandular trichomes of the plant (Hammond and Mahlberg, 1977). Over 70 cannabinoids have been isolated from *Cannabis sativa*, the major biologically active compound being Δ^9- tetrahydrocannabinol, commonly referred as THC (Mechoulam and Ben-Shabat, 1999). Besides its psychoactivity, THC possesses analgesic, anti-inflammatory, appetite stimulant and anti-emetic properties making this cannabinol a very promising therapeutic drug, especially for cancer and AIDS patients (Sirikantaramas *et al*., 2005). The pharmacologic and therapeutic potency of preparations of *Cannabis sativa* L. and its main active constituent Δ^9-tetrahydrocannabinol (THC) has been extensively reviewed by researchers (Mechoulam, 1986; Formukong *et al*., 1989; Grinspoon and Bakalar, 1993; Mattes *et al*., 1993; 1994; Brenneisen *et al*., 1996).

THC has a tremendous commercial value in the pharmaceutical market. Since *C. sativa* is a natural and inexpensive source of THC (as compared to producing it synthetically), efforts to select *Cannabis* varieties with high THC content are underway. However, due to the allogamous (cross fertilization) nature of the species, it is very difficult to maintain the chemical profile of selected high THC-producing genotypes under field conditions. Since this plant is also used as an illicit drug, its cultivation in open field must be done in secured areas and is highly regulated in the USA and some other parts of the world. Considering these limitations, indoor cultivation of a selected high yielding genotype/clone under controlled environmental conditions is the most suitable way to maintain its potency and efficacy while circumventing the regulatory problems. The objective of this study was to determine the effect of light intensity, temperature and CO_2 conditions on gas and water vapour exchange characteristics of *C. sativa* L. to establish suitable and efficient environmental conditions for its indoor cultivation.

MATERIAL AND METHODS

To study the photosynthetic response of *C. sativa* under different PPFD and temperature levels, leaves of twenty vegetatively propagated, four month old plants from a single mother plant of high yielding Mexican variety were exposed to a range of PPFD (0, 500, 1000, 1500 and 2000 μmol $m^{-2}s^{-1}$) and temperature conditions (20, 25, 30, 35 and 40 ^oC) under controlled humidity (55 ± 5 %) and CO_2 (350 ± 5 μmol mol^{-1}) concentration to determine suitable environmental conditions for it's optimum photosynthetic assimilation. Thereafter, leaves were acclimated under optimum light and temperature conditions and exposed to different CO_2 concentrations (250, 350, 450, 550, 650 and 750 μmol mol^{-1}) to study the effect of CO_2 on photosynthetic and water vapour characteristics of this species. All the measurements were carried out on five upper undamaged, fully expanded and healthy leaves of each plant with the help of a closed portable photosynthesis system (Model LI-6400; LI-COR, Lincoln, Nebraska, USA) equipped with light, temperature, humidity and CO_2 controls. Different PPFD were provided with the help of an artificial light source (Model LI-6400-02; light emitting silicon diode; LI-COR), fixed on the top of the leaf chamber and were recorded with the help of quantum sensor kept in range of 660-675 nm, mounted at the leaf level. The rate of dark respiration was measured by maintaining the leaf cuvette at zero irradiance. To avoid any radiation from

outside the leaf chamber was covered with a black cloth through the respiratory measurements. Temperature of the cuvette was controlled by the integrated Peltier coolers, which is controlled by the microprocessor. Different concentrations of CO_2 were supplied to the cuvette of climatic unit (LI-6400-01, LI-COR Inc., USA) by mixing pure CO_2 with CO_2 free air and were measured by infrared gas analyzer. All the measurements for gas and water vapour exchange were first recorded at lowest PPFD and temperature condition and then subsequently to the increasing levels of these parameters. Similarly, leaves under optimum PPFD and temperature conditions were first exposed to the lowest level of CO_2 concentration followed by elevated levels. Air flow rate (500 mmol s^{-1}) and relative humidity (55 ± 5%) were kept nearly constant throughout the experiment. Since steady state photosynthesis is reached within 30–45 min, the leaves were kept for about 45–60 min under each set of light conditions before the observations were recorded. Four gas exchange parameters viz., photosynthetic rate (P_N), transpirational water loss (E), stomatal conductance for CO_2 (g_s) and intercellular CO_2 concentration (Ci) were measured simultaneously at steady state condition under various permutations and combinations of light and temperature. Water use efficiency (WUE) was calculated as a ratio of the rate of photosynthesis and transpiration. A correlation and multiple regression analysis of data was performed on the basis of multiple linear hypothesis P_N, E, g_s, Ci, Ci/Ca and WUE as a dependent variable on PPFD, temperature and different CO_2 concentrations using SYSTAT-11 (Systat Software Inc. San Jose, CA, USA) statistical software.

RESULTS AND DISCUSSION

Both photosynthetic assimilation and biomass production are temperature- and light-dependent processes. The potential for photosynthetic acclimation to growth temperature is quite variable between species. Generally, variations in P_N reflect adjustment to the respective growth environment and also to the resistance to climate rigors. Although plants can exhibit a high degree of plasticity with respect to temperature response of photosynthesis, there is a general consensus that the optimum temperature for photosynthesis for an individual plant species reflects the environmental temperature range for which the species is genetically and physiologically adapted (Berry and Bjorkman, 1980). On other hand, response of photosynthesis to PPFD has been a long standing interest. At the leaf surface, low PPFD might be a limiting factor and high PPFD may be a threat to the plant metabolism if the irradiance exceeds the demand of photosynthesis (Osmond, 1994; Aguirre-von Wobeser *et al.*, 2000). Therefore, determination of the conditions for optimum gas and water vapour exchange processes is a prerequisite for growing any species indoor. According to our data on *C. sativa*, temperature optima for P_N was observed at 30 ºC. In general, temperature higher than 30 ºC had an adverse effect on P_N (Fig. 1A). At 25 ºC, rate of photosynthesis increased with increasing PPFD, but this trend peaked with 1500 μmol $m^{-2}s^{-1}$ PPFD at 30 ºC, and decreased at higher light intensities. Similar effect of

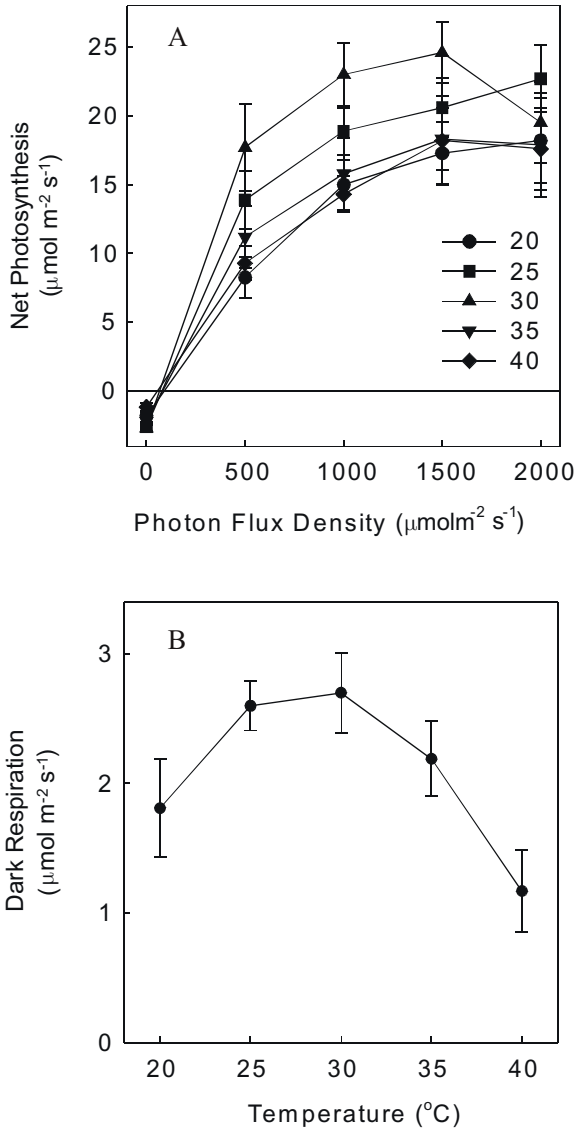

Fig. 1. A. Variations in net photosynthesis in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions. B. The temperature dependence of Dark respiration in *Cannabis sativa*.

PPFD was observed at temperatures higher than 30 °C. Maximum rate of photosynthesis ($P_{N\ max}$) was 24.60 μmol $m^{-2}s^{-1}$ at 30 °C and under 1500 μmol $m^{-2}s^{-1}$ PPFD. The interaction of PPFD and temperature demonstrates that high PPFD and higher temperature together (PPFD × temperature) had an adverse effect on P_N. In general, effect of PPFD (r = 87) was more prominent in regulating P_N in *Cannabis sativa* as compared to temperature (r = 46).

An increase in Rd (μmol $m^{-2}s^{-1}$ PPFD) was observed with increasing temperature up to 30 °C and decreased at higher temperature (Fig. 1B). Working on two different populations of *Podophyllum hexandrum*, Singh and Purohit (1997) reported a linear increase in Rd with temperature (up to 40 °C) in alpine population whereas; in temperate population, Rd increased with temperature up to 30 °C and decreased at higher levels. 2 to 10 fold increase in Rd was reported by Joshi and Palni (1998) in different tea leaves with increase in temperature from 20 to 40 °C; higher temperature however, was associated with clones having higher photosynthetic rates. In *C. sativa*, decrease in Rd followed a trend similar to P_N, with varying temperatures. Reduced P_N, and increased Rd are reported to limit the productivity in some plant species at higher temperatures (Alexander *et al*., 1995; Thornton *et al*., 1995).

Stomatal conductance was commensurate to PPFD levels, irrespective of temperature (Fig. 2). A positive correlation (r = 56) was observed between PPFD and g_s in *C. sativa*. On other hand, g_s increased with increasing temperature up to a maximum value at 30 °C and decreased at higher temperatures under all the PPFD labels. Maximum value of g_s was recorded at 30 °C and highest level of PPFD (2000 μmol $m^{-2}s^{-1}$).

In contrast to g_s, E increased in response to both higher temperature and high PPFD. Lowest value of E (2.38 ± 0.28 mmol $m^{-2}s^{-1}$) was observed at 20 °C under 0 μmol $m^{-2}s^{-1}$ PPFD, whereas highest value (7.60 ± 0.33 mmol $m^{-2}s^{-1}$) was recorded at 40 °C under 2000 μmol $m^{-2}\ s^{-1}$ (Fig. 3). Transpiration rate is known to depend on g_s (Alexander *et al*., 1995), and it seems to be major factor driving E in the present study. An increase in E and decrease in g_s is reported in many plant studies (Rawson *et al*., 1977; Schulze *et al*., 1972).

Intercellular CO_2 concentration (Ci) decreased with increase in PPFD and temperatures up to highest level tested (PPFD up to 2000 μmol $m^{-2}s^{-1}$ and temperature up to 40 °C (Fig. 4). Highest Ci (367 ml L^{-1}) was observed at lowest PPFD and temperature conditions i.e. 20 °C and 0 μmol $m^{-2}s^{-1}$ PPFD and, thereafter lowest Ci (149 ml L^{-1}) was recorded at highest PPFD and temperature conditions. However, the decrease was less pronounced at light intensities above 1500 μmol $m^{-2}s^{-1}$. Effect of temperature on depression of Ci was more prominent above 30 °C. Higher temperature and higher light together had a significant adverse effect on Ci of this species. Photosynthetic data particularly on Ci and g_s,

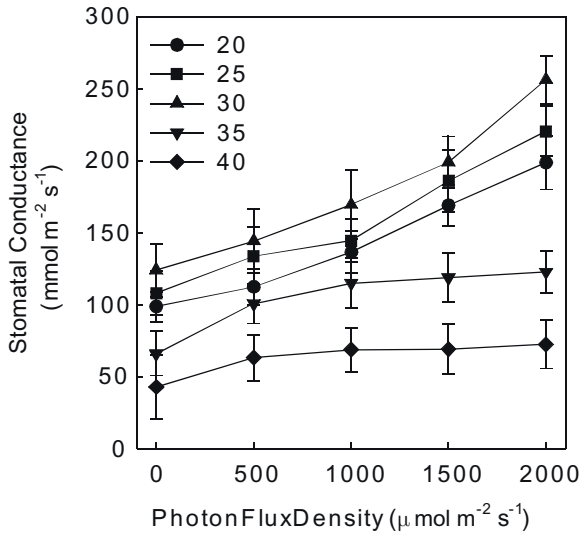

Fig. 2. Variations in stomatal conductance in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.

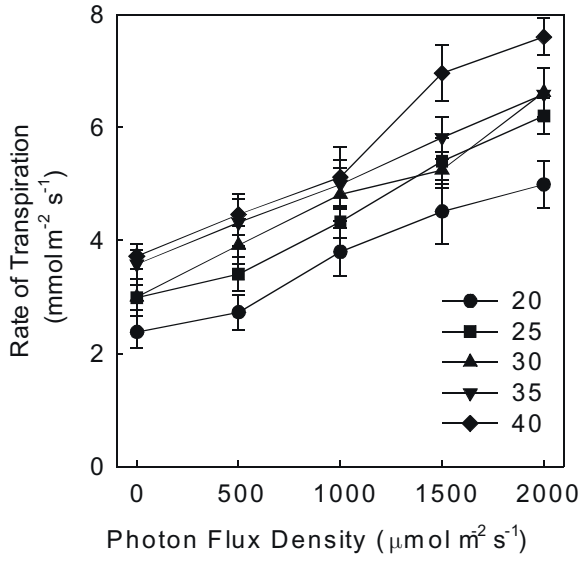

Fig. 3. Variations in rate of transpiration in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.

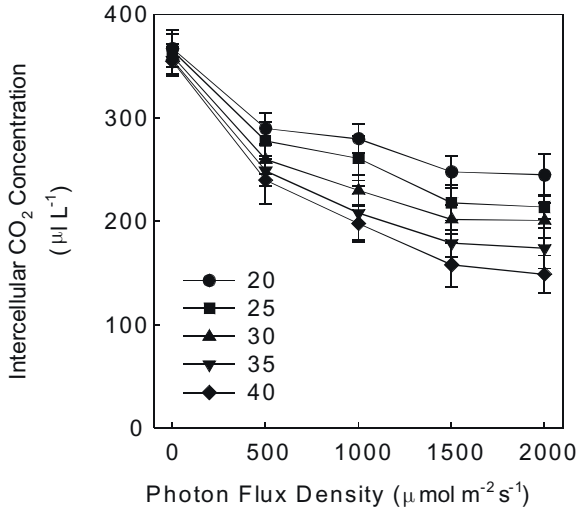

Fig. 4. Variations in intercellular CO_2 concentration in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.

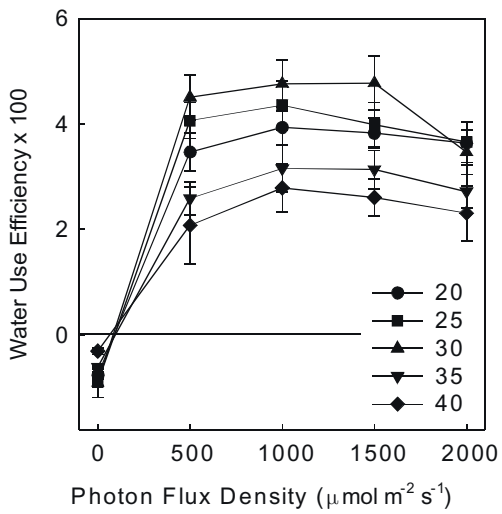

Fig. 5. Variations in water use efficiency in *C. sativa* with varying photosynthetic photon flux densities (PPFD) and temperature conditions.

indicates that both stomatal and mesophyll factors seems to be involved in the mechanism of control of photosynthesis by temperature and light in *C. sativa*.

Similar to Ci, a gradual decrease in Ci/Ca ratio was also observed with increasing PPFD and temperature conditions (Table 1). About 32 %, 41 %, 44 %, 50 % and 57 % decrease in Ci/Ca ratio was observed at 20, 25, 30, 35 and 40 °C respectively when plants were exposed from 0 to 2000 μmol $m^{-2}s^{-1}$ PPFD. Similarly, about 3 %, 17 %, 29 %, 37 % and 39 % depression was observed under 0, 500, 1000, 1500 and 2000 μmol $m^{-2}s^{-1}$ PPFD when plants were exposed to 40 °C as compared to 25 °C. Although essentially a biochemical process, photosynthesis is often regarded as a diffusive process. The rate of diffusion of CO_2 is largely controlled by two factors, g_s and CO_2 concentration gradient between carboxylation site and ambient air (Ca). This CO_2 concentration gradient at given g_s and Ca is established predominantly by Ci, which is a result of mesophyll efficiency. Therefore, the diffusive entry of CO_2 into leaf is a reflection of intrinsic mesophyll capacity. Sheshshayee *et al*. (1996) have reported Ci/g_s ratio as an indicator of mesophyll efficiency and a representation of mesophyll control on P_N. Our data also represent highest mesophyll efficiency (i.e. lowest Ci/g_s ratio) around 30 °C and 1500 μmol $m^{-2}s^{-1}$ PPFD. Values of Ci/g_s ratio increased with temperature higher than 30 °C, which further confirms that a combination of 30 °C temperature and 1500 μmol $m^{-2}s^{-1}$ PPFD may be best suitable for the indoor cultivation of *C. sativa*.

Table 1. Effect of different photosynthetic photon flux density and temperature conditions on Ci/Ca ratio in the leaves of *Cannabis sativa*.

Light Intensities (μmol $m^{-2}s^{-1}$)	Temperature (^{0}C)				
	20	25	30	35	40
000	1.04 ± 0.12	1.04 ± 0.14	1.02 ± 0.11	1.01 ± 0.09	1.01 ± 0.07
500	0.82 ± 0.05	0.79 ± 0.06	0.74 ± 0.06	0.71 ± 0.06	0.68 ± 0.05
1000	0.80 ± 0.06	0.75 ± 0.04	0.66 ± 0.06	0.59 ± 0.04	0.57 ± 0.06
1500	0.71 ± 0.04	0.62 ± 0.06	0.58 ± 0.05	0.51 ± 0.05	0.45 ± 0.04
2000	0.70 ± 0.06	0.61 ± 0.05	0.57 ± 0.05	0.50 ± 0.04	0.43 ± 0.03

Table 2. Effect of different levels of CO_2 on net photosynthesis (P_N), transpiration (E), stomatal conductance (g_s), internal CO_2 concentration (Ci), Ratio of internal to external CO_2 concentration (Ci/Ca) and water use efficiency (WUE) on the leaves of *Cannabis sativa*.

CO_2 levels (μmol mol^{-1})	P_N (μmol CO_2 m^{-2}s^{-1})	E (mmol H_2O m^{-2}s^{-1})	g_s (mmol CO_2 m^{-2}s^{-1})	Ci (μl L^{-1})	Ci/Ca ratio	WUE × 100
250	12.48 ± 1.76	5.69 ± 0.47	202.76 ± 19.78	138.00 ± 11.42	0.55	2.19
350	24.64 ± 2.24	5.31 ± 0.35	195.99 ± 18.40	202.00 ± 14.00	0.47	4.64
450	24.76 ± 1.89	5.76 ± 0.44	189.78 ± 16.97	260.00 ± 19.34	0.58	4.30
550	26.54 ± 2.12	4.87 ± 0.38	148.37 ± 13.99	330.00 ± 22.47	0.60	5.46
650	30.48 ± 2.76	4.65 ± 0.76	136.08 ± 12.36	385.00 ± 33.24	0.61	6.56
750	36.80 ± 3.18	3.75 ± 0.33	112.76 ± 10.32	435.00 ± 37.23	0.58	9.81

At 20 and 25 °C, WUE increased with increase in PPFD up to 2000 μmol m^{-2}s^{-1} (Fig. 5). On the other hand, WUE increased only up to 1500 μmol m^{-2}s^{-1} PPFD at 30 °C and decreased thereafter at higher light levels. Temperature higher than 30 °C had an adverse effect on WUE of this species. The maximum WUE was observed at 30 °C and under 1500 μmol m^{-2}s^{-1} PPFD. Photosynthesis appears to have a greater influence than E over regulating water use efficiency in *C. sativa*. A highly significant positive correlation was observed between WUE and P_N (r = 0.92). Together, high temperature and high PPFD had an adverse effect on the WUE in *C. sativa*.

Increasing atmospheric CO_2 is a global environmental concern. Atmospheric CO_2 has risen from pre- industrial value of ~ 280 μmol mol^{-1} to present concentration of ~ 372 μmol mol^{-1} and is expected to exceed 700 μmol mol^{-1} by the end of century (Prentice *et al*., 2001; Long *et al*., 2004). Since ambient CO_2 concentration as a substrate is still a limiting factor for photosynthesis in C_3 plants, attempts are being made to study how changes in atmospheric CO_2 concentration will affect crops (Bowes, 1993; Drake *et al*., 1997; Long *et al*., 2004). This study on *Cannabis sativa* shows that P_N, WUE and Ci decreased by 50 %, 53 % and 10 % respectively, and Ci/Ca, E and g_s increased by 25 %, 7 % and 3 %, respectively, when measurements were made at 250 μmol mol^{-1} as compared to ambient CO_2 (~350 μmol mol^{-1}) level (Table 2). An average of 30 to 33 % increase in P_N and productivity of C_3 plants with doubling atmospheric CO_2 concentration has been already reported by Kimball 1983a, b; 1986; Idso and Idso 1994; Bazzaz and Gabutt, 1988; Cure and Acock, 1986. In *C. sativa*, a doubling of CO_2 concentration (750 μmol mol^{-1}) suppressed E and g_s ~29 % and 42 % respectively, and stimulated P_N, WUE and Ci by 50%, 111 % and 115 % respectively as compared to ambient CO_2 concentration. Doubling CO_2 level had a significant effect on all these parameters. Suppression in g_s and consequently in E (Emaus *et al*., 1993; Thomas *et al*., 1994) and improvement in P_N and WUE and Ci (Kimball 1983a, b; 1986; Idso and Idso 1994, Morison, 1993) under elevated CO_2 concentration is reported in many other plant species. Higher WUE under elevated CO_2, primarily because of decreased g_s and E, may enable this species to survive under drought conditions. This species maintained nearly constant values of Ci/Ca with increasing CO_2 concentration despite the increase in P_N and WUE, and decrease in g_s and E, represents a close coordination between stomatal and mesophyll functions (Morison, 1993) and reported to improve growth and productivity of plant (Jones, 1992).

In view of our results, it is concluded that *C. sativa* can utilize a fairly high level of PPFD and temperature for its gas and water exchange processes, and can perform much better if grown at ~ 1500 μmol m^{-2} s^{-1} PPFD and around 25 to 30 °C temperature conditions. Furthermore, higher P_N, WUE and nearly constant Ci/Ca ratio under elevated CO_2 concentration, reflects its potential for improved growth and productivity in drier and CO_2 rich environment.

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